MicroBioTest Protocol

Efficacy Evaluation of Continuous Bacterial Contamination Reduction on Enhanced Hard Surfaces as a Sanitizer - Supplemental

Testing Facility
MicroBioTest
Division of Microbac Laboratories, Inc.
105 Carpenter Drive
Sterling, VA 20164

Prepared for
Luminore, Inc.
6060 Corte del Cedro
Carlsbad, CA 92011

June 16, 2014

MicroBioTest Protocol: LUM.5a.06.16.14

MicroBioTest Project: 872 - 110

OBJECTIVE:

This test is designed to substantiate effectiveness claims for a substance containing copper with sanitizing claims intended to be registered with the Environmental Protection Agency as an inanimate hard surface other than those that come in contact with food or beverages. The test is consistent with the EPA Test Method for the Continuous Reduction of Bacterial Contamination on Copper Alloy Surfaces.

TESTING CONDITIONS:

A total of five replicates per challenge microorganism will be evaluated using carriers prepared from the copper enhanced hard surface. Two lots of the test surface will be evaluated. Prepared carriers of the test surface will be inoculated and re-inoculated based on the required regimen with *Pseudomonas aeruginosa*, Methicillin Resistant *Staphylococcus aureus*, and *Escherichia coli* O157:H7, held for the stipulated contact time(s), transferred to a neutralizing solution and mixed. Dilutions of the neutralizer will be plated, incubated and observed for growth.

MATERIALS:

A. Test and control surfaces supplied by the sponsor: (see last page for details).

Test and control carriers: 1" x 1" coupons, also referred to as carriers

- The identity, strength, purity, and composition, or other characteristics which will appropriately define the test, control, or reference surfaces shall be determined for each batch and shall be documented by the sponsor before its use in a study. Methods of synthesis, fabrication, or derivation of the test, control, or reference surfaces shall be documented and retained by the sponsor.

- When relevant to the conduct of the study the solubility of each test, control, or reference agent shall be determined by the sponsor before the experimental start date. The stability of the test, control, or reference agent shall be determined by the sponsor before the experimental start date or concomitantly according to written standard operating procedures, which provide for periodic analysis of each batch.

The test and control surfaces will be tested as supplied by the sponsor unless directed otherwise. All operations performed on the surfaces such as dilution or specialized storage conditions must be specified by the sponsor before initiation of testing.

The sponsor assures MicroBioTest, Division of Microbac Laboratories, Inc. (MicroBioTest) testing facility management that the test surface has been appropriately tested for identity, strength, purity, stability, and uniformity as applicable.

MicroBioTest will retain all unused test and control surfaces after completion of the test, and then only discard them with client permission in a manner that meets the approval of the safety officer.

B. Materials supplied by MicroBioTest including but not limited to:

1. Challenge microorganisms, required by EPA and the sponsor:
   a. *Pseudomonas aeruginosa*, ATCC 15442
   b. Methicillin Resistant *Staphylococcus aureus* (MRSA), ATCC 33592
   c. *Escherichia coli* O157:H7, ATCC 35150

2. Media and reagents:
   a. Tryptic Soy Broth (TSB)
   b. Neutralizer: 2X Letheen Broth
   c. Phosphate Buffer Saline dilution blanks (PBS)
   d. Tryptic Soy Agar (TSA)
   e. Heat-inactivated Fetal Bovine Serum (FBS)
   f. Triton X-100 solution (1% solution)
   g. Sterile deionized water
   h. 70-85% Isopropyl alcohol

3. Miscellaneous laboratory equipment and supplies.

4. Media, reagents and supplies for Antimicrobial Susceptibility Testing of MRSA:
   a. TSA containing 5% defibrinated sheep's blood (TSA+)
   b. 0.85% NaCl (SS)
   c. Mueller Hinton Agar (MHA)
   d. Control microorganism: *Staphylococcus aureus*, ATCC 25923

e. 0.5% McFarland Standard
f. Caliper measuring device
g. 1 µg Oxacillin disc

TEST SYSTEM IDENTIFICATION:

All test and control tube racks will be labeled with microorganism, test agent (if applicable) and project number prior to initiation of the study and during incubation. Petri dishes will be labeled with microorganism prior to initiation of the study and microorganism and project number during incubation.

EXPERIMENTAL DESIGN:

A. Inocula preparation:

Bacteria from stock cultures will be transferred into TSB and incubated at 35-37°C for 24±2 hours. Daily transfers will be made for at least three consecutive days (but no more than 10 days). For each transfer, tubes containing 10 mL of TSB will be inoculated using two loopfuls (4-mm inside diameter) of inoculum for each tube. A 48±4 hour culture will be used for the inocula on the day of testing.

The pellicle formed in the *Pseudomonas aeruginosa* culture will be aspirated before use.

Transfers more than 15 days away from the stock cultures will not be used for the inocula for the test.

For each microorganism, each culture will be thoroughly mixed on a vortex-mixer and allowed to settle. The upper two-thirds of each culture will be aspirated and used as the inoculum.

B. Addition of organic load:

To each prepared inocula, a 0.25 mL aliquot of FBS plus 0.05 mL1% Triton X-100 solution to 4.70 mL of bacteria suspension to yield a 5% FBS and 0.01% Triton X-100 soil load.

C. Test and Control Carrier preparation:

The test and control surfaces will be cleaned by submersion in 70-85% in Isopropyl alcohol, rinsed with sterile deionized water, and allowed to air dry. After drying completely, the carriers may be steam sterilized for 15 minutes at 121°C, or sterilized in a method approved by the sponsor. The carriers will be allowed to cool and held at ambient room temperature until use. Prior to use, each carrier will be aseptically transferred into plastic Petri dishes (one dish for each carrier) matted with two pieces of filter paper using sterile forceps.

For each lot of the test material, per microorganism, five sets with five replicate carriers per set will be prepared along with five sets per microorganism of the control material with three replicate carriers each for the primary aspects of the test. Additional surfaces will be prepared as required for remaining controls.

Table 1: Test and Control Carrier Description and Count

Method	Lot	Test and Primary Controls				Miscellaneous Controls				Grand Total
		Reps	Organisms	Contact Time or Runs	TOTAL	NE	Viab	Sterility	TOTAL	
Continuous Reduction	1	5	3	5	75	3	0	1	4	79
	2	5	3	5	75	3	0	1	4	79
	3	5	3	5	75	3	0	1	4	79
	Control	3	3	5	45	3	3	1	7	52

D. Test:

All test surfaces will be inoculated at staggered intervals with 5 µl of the challenge microorganism using a calibrated pipette. The inoculum will be spread to within approximately 1/8" of the edge of the carrier. This initial inoculation will be considered as "time zero".

The carriers will be dried at ambient conditions for the duration of exposure. The exposure period(s) begins with the initial "time-zero" inoculation.

The applicable sets not removed for quantitative recovery (see below) will be re-inoculated in the same manner at 3, 6, 9, 12, 15, 18, and 21 hours post "time-zero" inoculation.

The applicable sets for quantitative recovery will be removed at 2 (single inoculation), 6 (two inoculations), 12 (four inoculations), 18 (six inoculations), and 24

(8 inoculations) hours. At the conclusion of the applicable contact time for each set of surfaces, each carrier will be transferred to a jar containing 20 mL of neutralizer at the appropriate staggered intervals. Each jar will be sonicated for five minutes and then rotated by hand to mix. Within one hour after sonication, serial dilutions will be prepared using PBS (10^{-1} – 10^{-4}). Duplicate 1.0 mL aliquots from each jar/dilution (10^{0} – 10^{-4}) will be plated using TSA pour plates.

Plates will be incubated for 48±4 hours at 35-37°C, colonies will be counted and CFU/carrier calculated.

E. Controls:

1. Carrier quantitation control:

   For each challenge microorganism, a parallel control will be run using the control carriers (surfaces) in the same manner as the test (inoculation and quantitative recovery) with the exception that three replicates will be evaluated rather than five. All plates will be incubated appropriately in the same manner as the test plates.

2. Culture purity control:

   Each prepared culture will be streaked for isolation using TSA. All plates will be incubated in the same manner as the test plates. The isolated cultures will be observed for purity.

3. Organic soil sterility control:

   Duplicate 1.0 mL aliquots of the prepared organic soil will be plated in TSA pour plates. The plates will be incubated with the test plates observed for growth or no growth.

4. Inoculum confirmation counts control:

   Each prepared inoculum will be serially diluted using PBS and selected dilutions will be plated in duplicate using TSA pour plates. All plates will be incubated with the test plates.

5. Neutralizer sterility control:

A single jar of containing the neutralizer will be incubated with the test plates. The neutralizer will be observed for growth or no growth.

6. Carrier sterility control:

An uninoculated test (per lot) and control carrier will be subcultured into independent jars containing the neutralizer and incubated with the test plates. The neutralizer will be observed for growth or no growth.

7. Carrier viability control:

For each challenge microorganism, a single inoculated control carrier will be subcultured into a jar containing the neutralizer and incubated with the test plates. The neutralizer jars will be observed for growth or no growth.

8. Neutralizer effectiveness control:

For each challenge microorganism, per lot of the test article, a single sterile test carrier will be neutralized in the same manner as the test (transferred into individual jars containing 20 mL of neutralizer. To each jar, a 1.0 mL aliquot of the diluted inoculum will be added to yield ≤100 CFU/mL in the neutralizer. The jar will be mixed and a 1.0 mL aliquot will be removed and plated in duplicate.

A numbers control will be performed in the same manner with the exception that a sterile control carrier will be used.

All plates will be incubated with the test plates.

9. Antimicrobial Susceptibility Testing of MRSA:

The prepared MRSA culture will be subcultured onto a TSA+ plate and the plate will be incubated for approximately 24 hours at 35-37°C. Following incubation, a suspension will be prepared by suspending growth from the TSA+ culture in SS to yield equivalent turbidity to a 0.5 McFarland Standard. This prepared suspension will be streaked onto MHA plate in a cross-hatch pattern and a 1 µg Oxacillin disc will be placed onto the center of the plate. The plate will be inverted and incubated for ≥ 24 hours at 35-37°C.

The same procedures will be conducted concurrently using the control microorganism, *Staphylococcus aureus*, ATCC 25923 to confirm the validity of the assay.

The interpretation of the zone of inhibitions (ZOI) will be based on established National Committee for Clinical Laboratory Standards (NCCLS) performance standards. As currently published, (NCCLS standard M100-S21) ZOI breakpoints must be ≤ 10 mm (rounded to the nearest whole mm) confirms resistance, 11-12 mm is considered intermediate resistance, and ≥ 13 mm confirms susceptibility.

10. Microorganism confirmation procedures:

A randomly selected colony from the carrier quantitation control plates, and if applicable, a randomly selected colony from a test plate will be confirmed by colony morphology and Gram stain according to extant SOPs. The same procedures will be performed using the culture purity control plates and the result regarding purity will be documented as well.

TEST ACCEPTANCE CRITERIA:

The test will be acceptable for evaluation of the test results if the neutralizer is effective and non-toxic. The study director may consider other causes that may affect test reliability and acceptance. There are no proposed statistical methods for this test.

- The average recovery for the Carrier Quantitation Control must be at least 2.0×10^4 CFU/carrier (for each quantitative recovery period).
- The CFU recovered for the neutralizer effectiveness controls should be within 1.0 $\log_{10}$ of the parallel neutralization confirmation control.
- The carrier sterility controls must exhibit no growth.
- The carrier viability controls must exhibit growth.
- The purity controls must demonstrate pure cultures.
- The organic soil sterility control must exhibit no growth.
- The neutralizer sterility control must exhibit no growth.
- For the Antimicrobial Susceptibility Testing: the test MRSA strain must exhibit resistance and the *Staphylococcus aureus* control strain (ATCC 25923) must exhibit susceptibility to Oxacillin.

PRODUCT EVALUATION CRITERIA:

According to EPA guidelines, the test agent meets effectiveness requirements, if the test results exhibit a minimum bacterial reduction of at least 90% over the corresponding Carrier Quantitation Controls at all recovery times over the 24 hour inoculation and exposure period.

DATA PRESENTATION:

The final report will include the following information in tabular form:

- The average colony-forming units (CFU)/carrier and percent reduction for each evaluation.
- The results for all the controls.

PERSONNEL AND TESTING FACILITIES:

A study director will be assigned before initiation of the test. Resumes for technical personnel are maintained and are available on request. This study will be conducted at MicroBioTest, 105 Carpenter Drive, Sterling, VA 20164.

CONFIDENTIALITY:

All data generated at MicroBioTest are held in strictest confidence and are available only to the sponsor and the sponsor designated authorities (if applicable). In turn, no reference to MicroBioTest's promotion of the evaluated test articles may be made public by the sponsor.

REPORT FORMAT:

MicroBioTest employs a standard report format for each test design. Each final report provides the following information:

- Sponsor identification and test agent identification
- Type of test and project number
- Dates of study initiation and completion
- Interpretation of results and conclusions

- Test results
- Methods and evaluation criteria
- Signed Quality Assurance and Compliance Statements (for GLP studies, if provided by the sponsor)

REGULATORY COMPLIANCE AND QUALITY ASSURANCE (applicable to GLP studies only)

This study will be performed in compliance with the US Environmental Protection Agency's Good Laboratory Practices regulations, 40 CFR 160. Note: information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study unless otherwise stated.

The Quality Assurance Unit of MicroBioTest will inspect the conduct of the study for GLP compliance. The dates of the inspections and the dates that findings are reported to the study management and study director will be included in the final report.

RECORDS TO BE MAINTAINED:

All raw data, protocol, protocol modifications, test agent records, final report, and correspondence between MicroBioTest and the sponsor will be stored in the archives at MicroBioTest, 105 Carpenter Drive, Sterling, Virginia 20164 or in a controlled facility off site.

All changes or revisions to this approved protocol will be documented, signed by the study director, dated and maintained with this protocol. The sponsor will be notified of any change, resolution, and impact on the study as soon as practical.

The proposed experimental start and termination dates; additional information about the test agent; challenge microorganism used; media and reagent identification; and the type of neutralizers employed in the test will be addressed in a project sheet issued separately for each study. The date the study director signs the protocol will be the initiation date. All project sheets will be forwarded to the study sponsor.

MISCELLANEOUS INFORMATION:

The following information is to be completed by sponsor before initiation of study:

A. Name and address: Luminore, Inc.
6060 Corte del Cedro
Carlsbad, CA 92011

B. Test surface information:

Test surface name	LuminOre Copper-Nikel	
Lot No.	Lot 1	Lot 2
	092314A	092414A
- Manufacture Date	09/23/2014	09/24/2014
- Expiration Date	NA	NA
Active ingredient	Copper/Nickel	
Substrate	aluminum magnesium (the sponsor will provide coupons that are not treated with the active ingredient for use as the control carriers as applicable)	

Note: both lots will be tested (therefore supplied) at or below the Lower Certified Limit (LCL)

MISCELLANEOUS INFORMATION: (continued)

C. Test conditions:

Inoculation intervals: "0 time", 3, 6, 9, 12, 15, 18, and 21 hours

Evaluated contact times: 2, 6, 12, 18, and 24 hours

Exposure temperature: Ambient room temperature 20±1C

D. Organic load – serum added to achieve 5% in the inoculum: ■ yes ☐ no

E. Precautions/storage – MSDS or certificate of analysis provided: ☒ yes ☐ no

REPORT HANDLING: The sponsor intends to submit this information to: US EPA

STUDY CONDUCT: GLP

PROTOCOL APPROVAL:

Sponsor:
LuminOre, Inc.
A California Corporation

By: Thomas J. Valente Date: OCT 8, 2014
Thomas J. Valente

Title: President

Study Director Signature: Date: 11/15/14
Angela L. Hollingsworth

Date Issued: 11/15/14	Project Sheet No. 1	Page No. 1	Laboratory Project Identification No. 872-110

STUDY TITLE: Efficacy Evaluation of Continuous Bacterial Contamination Reduction on Enhanced Hard Surfaces as a Sanitizer - Supplemental

STUDY DIRECTOR: Angela L. Hollingsworth

Signature 11/15/14 Date

TEST AND CONTROL ARTICLES:	LOT NO:	DATE RECEIVED:	DS NO:
LuminOre Copper/Nickel	092314A	10/17/14	E529
LuminOre Copper/Nickel	092414A	10/17/14	E530
Non Treated Coupons	Not applicable	10/17/14	E532

PERFORMING DEPARTMENT: Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: K4
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

PROTECTIVE PRECAUTION REQUIRED: MSDS ☐ Yes / ■ No (Certificate of Analysis was provided)

PHYSICAL DESCRIPTION: ■ Solid ☐ Liquid ☐ Aerosol ☐ Other:

PURPOSE: See attached protocol. **AUTHORIZATION:** See client signature.

PROPOSED EXPERIMENTAL START DATE: 11/16/14 **TERMINATION DATE:** 11/19/14

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:

SPONSOR: Luminore, Inc.
6060 Corte del Cedro
Carlsbad, CA 92011

CONTACT PERSON: Thomas J. Valente
Phone: (760) 431-7705 ext: 101
E-mail: tom@luminore.com

TEST CONDITIONS:

Challenge organism(s): *Pseudomonas aeruginosa*, ATCC 15442
Methicillin Resistant *Staphylococcus aureus*, ATCC 33592
Escherichia coli O157:H7, ATCC 35150

Active ingredient(s): Copper/Nickel

Neutralizer(s): Letheen Broth – 2X

Contact Time(s): 2, 6, 12, 18, and 24 hours

Contact Temperature(s): Ambient (20±1°C) Dilution(s): Ready to Use

Organic Load: ■ Yes / ☐ No (Per the protocol to achieve 5% in the inoculum)

Incubation Time(s): 48±4 hours

Incubation Temperature(s): 35-37°C

Comments: The sponsor provided aluminum magnesium coupons that are not treated with the active ingredient for use as the control carriers (Non Treated Coupons).

Date Issued: 02/04/15	Project Sheet No. 2	Page No. 1	Laboratory Project Identification No. 872-110

STUDY TITLE: Efficacy Evaluation of Continuous Bacterial Contamination Reduction on Enhanced Hard Surfaces as a Sanitizer - Supplemental

STUDY DIRECTOR: Angela L. Hollingsworth

Signature For ALH FEB 04 2015 Date

TEST AND CONTROL ARTICLES:	**LOT NO:**	**DATE RECEIVED:**	**DS NO:**
LuminOre Copper/Nickel	092314A	10/17/14	E529
LuminOre Copper/Nickel	092414A	10/17/14	E530
Non Treated Coupons	Not applicable	10/17/14	E532

PERFORMING DEPARTMENT:
Applied Microbiology Laboratory

STORAGE CONDITIONS: Location: K4
■ Dark ■ Ambient Room Temperature
☐ Desiccator ☐ Freezer ☐ Refrigerator ☐ Other:

CONDUCT OF STUDY: ☐ FDA ■ EPA ☐ R&D ■ GLP ☐ GCP ☐ Other:

SPONSOR:	Luminore, Inc. 6060 Corte del Cedro Carlsbad, CA 92011	**CONTACT PERSON:** Phone: E-mail:	Thomas J. Valente (760) 431-7705 ext: 101 tom@luminore.com

EXPLANATION:

Protocol Amendment(s):

1. Page 11 of the protocol did not specify the percent of copper in each test surface. Per the sponsor, the percentage of copper in each test surface is as follows:
   - Lot No. 100214A: 81.9% copper
   - Lot No. 100114A: 81.0% copper
   - Lot No. 100114B: 82.3% copper

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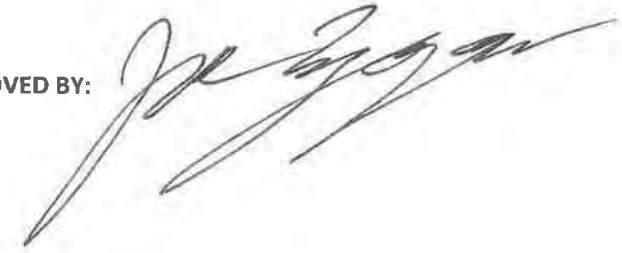
6060 Corte Del Cedro
Carlsbad, CA 92011
Phone: 760-431-7705
Fax: 760-431-7706

Certificate of Analysis

Material: Copper/Nickel
Batch No.: 092314A

Parameter	Actual	Acceptable
Temperature	82°F	No less than 70°F
Humidity	36%	Less than or equal to 40%
Cross link	Pass	Pass
Mil thickness	Pass	No less than 12 mils

Passes all quality control standards for the LuminOre product.

APPROVED BY: 

Date: 10-16-14

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visualize | realize | metalize

6060 Corte Del Cedro
Carlsbad, CA 92011
Phone: 760-431-7705
Fax: 760-431-7706

Certificate of Analysis

Material: Copper/Nickel
Batch No.: 092414A

Parameter	Actual	Acceptable
Temperature	81°F	No less than 70°F
Humidity	29%	Less than or equal to 40%
Cross link	Pass	Pass
Mil thickness	Pass	No less than 12 mils

Passes all quality control standards for the LuminOre product.

APPROVED BY: Jon Baker **Date:** 10-16-14